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This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

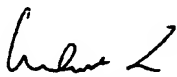
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<input type="checkbox"/> Additional inventors are being named on the _____ separately numbered sheets attached hereto					
TITLE OF THE INVENTION (280 characters max)					
PROCESS FOR PREPARATION OF FLAX PROTEIN ISOLATE					
Direct all correspondence to: CORRESPONDENCE ADDRESS					
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ENCLOSED APPLICATION PARTS (check all that apply)					
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Respectfully submitted,

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24,973

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This collection of information is required by 37 CFR 1.51. The information is used by the public to file (and by the PTO to process) a provisional application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the complete provisional application to the PTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, D.C. 20231. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Box Provisional Application, Assistant Commissioner for Patents, Washington, D.C.

P19LARGE/REV05

TITLE OF INVENTION

PROCESS FOR PREPARATION OF FLAX PROTEIN ISOLATE

FIELD OF INVENTION

[0001] The present invention relates to the recovery of flax protein isolates from flax oil seed meal.

BACKGROUND OF THE INVENTION

[0002] In US Patent Application No. 10/266,677 filed October 9, 2002, assigned to the assignee hereof and the disclosure of which is incorporated herein by reference, there is described the preparation of a flax protein isolate. As set forth therein, there is provided a flax oil seed protein isolate having a protein content of at least about 90 wt%, preferably at least about 100 wt%, as determined by Kjeldahl nitrogen x 6.25 (N x 6.25) on a dry weight basis.

[0003] In such process, yields of flax protein isolate were limited because of the inability to concentrate the protein solution to high protein contents owing to the presence of water-soluble mucilage. Flax seed mucilage is a gummy substance consisting substantially of polysaccharides.

[0004] The presence of the mucilage in protein products separated from flax oil seed meal by other processes makes it difficult to produce product with protein contents high enough to be classified as isolates.

SUMMARY OF THE INVENTION

[0005] It has now surprisingly been found that, if an initial extraction of the flax seed at elevated temperature using a mildly-alkaline solution of sodium bicarbonate to remove mucilage is effected, then a much higher concentration of concentrated aqueous protein solution can be produced, enabling improved yields of flax protein isolate to be obtained. In addition, a flax protein isolate can be produced from flax protein meal by isoelectric precipitation or by a micellar route.

[0006] The present invention provides a protein isolate of any flax oil seed and a low linolenic acid variant known as Linola[®] oil seed and a procedure for preparation of the same involving an initial extraction of the oil seed. A protein isolate is defined as a protein containing at least about 90 wt% protein at a Kjeldahl nitrogen conversion rate of N x 6.25. The term "protein content" as used herein refers to the quantity of protein in the protein isolate expressed on a dry weight basis.

[0007] Linola oil seed is a mutant of flax oil seed in which the fatty acid composition has been changed and linolenic acid (C18:3) has been substantially reduced from about 50% in conventional flax oil seed to about 2%, through traditional breeding procedures. These modifications were made to provide from the resulting Linola oil seed an edible polyunsaturated oil substantially similar to sunflower oil in fatty acid composition.

[0008] The flax protein isolate produced according to the process herein may be used in conventional applications of protein isolates, such as, protein fortification of processed foods, emulsification of oils, body formers in baked goods and foaming agents in products which entrap gases. In addition, the protein isolate may be formed into protein fibers, useful in meat analogs, may be used as an egg white substitute or extender in food products where egg white is used as a binder. The flax protein isolate may be used as nutritional supplements. Other uses of the flax protein isolate are in pet foods, animal feed and in industrial and cosmetic applications and in personal care products.

BRIEF DESCRIPTION OF DRAWINGS

[0009] Figures 1 and 2 are HPLC chromatograms of linola isolates, with Figure 1 being that for a PMM-derived linola protein isolate and Figure 2 being that for a supernatant-derived linola protein isolate.

GENERAL DESCRIPTION OF INVENTION

[0010] The initial extraction of the flax seed is effected using an aqueous solution, generally having a pH of about 6 to about 7.5, preferably at the natural pH of the aqueous solution of the alkaline material, at an elevated temperature of about 30° to about 70°C, preferably at about 50°C. The extraction of flax oil seed may be effected at seed to solution ratio of about 1:1 to about 1:20, preferably about 1:5 to about 1:10, using an aqueous solution containing about 0.2 to about 0.7 M mildly-alkaline material. Preferably, an aqueous solution of sodium bicarbonate having a concentration of about 0.5 M is used at about 50°C in a ratio of flax seed to solution of about 1:8.

[0011] After a first extraction of the oil seed, generally by mixing with stirring of the oil seed in the aqueous alkaline solution, for about 15 to about 60 minutes, preferably about 30 to about 60 minutes, the extraction is preferably repeated with fresh aqueous alkaline solution until no further mucilage is extracted from the oil seeds.

[0012] The extracted oil seeds then are processed to recover the oil and to provide an oil seed meal from which a flax protein isolate may be produced.

[0013] One procedure whereby the flax protein isolate may be formed from the flax oil seed meal is by isoelectric precipitation. Prior to effecting the initial removal of mucilage as provided herein, the applicants had not been able to produce a flax protein isolate by isoelectric precipitation processing of flax oil seed meal. Isoelectric precipitation is commonly used to prepare other protein isolates, for example, soy protein isolate.

[0014] In such isoelectric precipitation, the flax oil seed meal, or linola oil seed meal, is extracted with an aqueous alkaline solution, generally an aqueous sodium hydroxide solution having a pH of about 8 to about 12, preferably about 9 to about 11, at a temperature of about 0° to about 40°C, preferably about 15° to about 25°C, at a meal concentration of about 2.5 to about 10% w/v, preferably about 5% w/v.

[0015] Following extraction of the meal, residual meal is separated from the aqueous protein solution in any convenient manner, such as by employing vacuum filtration, followed by centrifugation and/or filtration to remove residual meal. The separated residual meal may be dried for disposal.

[0016] The flax protein solution is then acidified to a pH of about 3 to about 5, preferably about 4, using any convenient acid, such as hydrochloric acid, to cause the formation of a precipitate of flax or linola protein isolate. The precipitate is removed from supernatant and dried. The dried flax protein isolate has a high protein content, in excess of about 90 wt% (N x 6.25), preferably at least about 100 wt%.

[0017] Alternatively and preferably, the flax protein isolate is prepared following the procedure described in the aforementioned copending US Patent Application No. 10/266,677. The process may be effected in a series of batch operations or as a continuous or semi-continuous process.

[0018] The initial step of the process of producing the flax protein isolate according to the procedure of the aforementioned application, involves solubilizing proteinaceous material from flax oil seed meal. The proteinaceous material recovered from flax seed meal may be the protein naturally occurring in flax seed or the proteinaceous material may be a protein modified by genetic manipulation but

possessing characteristic hydrophobic and polar properties of the natural protein. The flax meal may be any flax meal resulting from the removal of flax oil from flax oil seed with varying levels of non-denatured protein, resulting, for example, from hot hexane extraction or cold oil extrusion methods. The removal of flax oil from flax oil seed usually is effected as a separate operation from the protein isolate recovery procedure described herein.

[0019] Protein solubilization is effected most efficiently by using a salt solution since the presence of the salt enhances the removal of soluble protein from the oil seed meal. The salt usually is sodium chloride, although other salts, such as, potassium chloride, may be used. The salt solution has an ionic strength of at least about 0.10 M, preferably at least about 0.15 M, generally up to about 2.0 M to enable solubilization of significant quantities of protein to be effected. As the ionic strength of the salt solution increases, the degree of solubilization of protein in the oil seed meal initially increases until a maximum value is achieved. Any subsequent increase in ionic strength does not increase the total protein solubilized. The ionic strength of the salt solution which causes maximum protein solubilization varies depending on the salt concerned and the oil seed meal chosen.

[0020] In view of the greater degree of dilution required for protein precipitation with increasing ionic strengths, it is usually preferred to utilize an ionic strength value less than about 1.0 and more preferably a value of about 0.15 to about 0.6.

[0021] In a batch process, the salt solubilization of the protein is effected at a temperature of above about 0°C and preferably up to about 35°C, preferably accompanied by agitation to decrease the solubilization time, which is usually about 10 to about 90 minutes. It is preferred to effect the solubilization to extract substantially the maximum amount of protein from the oil seed meal, so as to improve product yield. The upper preferred temperature limit of about 35°C is chosen since the process becomes uneconomic at higher temperature levels in a batch mode.

[0022] In a continuous process, the extraction of the protein from the flax oil seed meal is carried out in any manner consistent with effecting a continuous extraction of protein from the flax oil seed meal. In one embodiment, the flax oil seed meal is continuously mixed with a salt solution and the mixture is conveyed through a pipe or conduit having a length and at a flow rate for a residence time sufficient to effect the

desired extraction in accordance with the parameters described herein. In such continuous procedure, the salt solubilization step is effected rapidly, in a time of up to about 10 minutes, preferably to effect solubilization to extract substantially the maximum amount of protein from the flax oil seed meal. The solubilization in the continuous procedure preferably is effected at elevated temperatures, generally up to about 60°C or more.

[0023] The aqueous salt solution and the flax oil seed meal have a natural pH of about 5 to about 7 to enable a protein isolate to be formed by the micellar route, as described in more detail below. The optimal pH value for maximum yield of flax or linola protein isolate varies depending on the flax oil seed meal chosen.

[0024] At and close to the limits of the pH range, protein isolate formation occurs only partly through the micelle route and in lower yields than attainable elsewhere in the pH range. For these reasons, pH values of about 5.3 to about 6.2 are preferred.

[0025] The pH of the salt solution may be adjusted to any desired value within the range of about 4 to about 7 for use in the extraction step by the use of any convenient acid, usually hydrochloric acid, or alkali, usually sodium hydroxide, as required.

[0026] The concentration of oil seed meal in the salt solution during the solubilization step may vary widely. Typical concentration values are about 5 to about 15% w/v.

[0027] The protein extraction step with the aqueous salt solution has the additional effect of solubilizing fats which may be present in the flax seed meal, which then results in the fats being present in the aqueous phase.

[0028] The protein solution resulting from the extraction step generally has a protein concentration of about 5 to about 40 g/L, preferably about 10 to about 30 g/L.

[0029] The aqueous phase resulting from the extraction step then may be separated from the residual flax oil seed meal, in any convenient manner, such as by employing vacuum filtration, followed by centrifugation and/or filtration to remove residual meal. The separated residual meal may be dried for disposal.

[0030] Where the flax seed meal contains significant quantities of fat, then the defatting steps described in US Patents Nos. 5,844,086 and 6,005,076, assigned to the

assignee hereof and the disclosures of which are incorporated herein by reference may be effected on the separated aqueous protein solution and on the concentrated aqueous protein solution discussed below.

[0031] As an alternative to extracting the flax oil seed meal with an aqueous salt solution, such extraction may be made using water alone, although the utilization of water alone tends to extract less protein from the flax oil seed meal than the aqueous salt solution. Where such alternative is employed, then the salt, in the concentrations discussed above, may be added to the protein solution after separation from the residual flax oil seed meal in order to maintain the protein in solution during the concentration step described below.

[0032] The aqueous protein solution then is concentrated to increase the protein concentration thereof while maintaining the ionic strength thereof substantially constant. Such concentration generally is effected to provide a concentrated protein solution having a protein concentration of at least about 150 g/L, preferably at least about 250 g/L.

[0033] The concentration step may be effected in any convenient manner consistent with batch or continuous operation, such as by employing any convenient selective membrane technique, such as ultrafiltration or diafiltration, using membranes, such as hollow-fibre membranes or spiral-wound membranes, with a suitable molecular weight cut-off, such as about 300 to about 50,000 daltons, having regard to differing membrane materials and configurations, and, for continuous operation, dimensioned to permit the desired degree of concentration as the aqueous protein solution passes through the membranes.

[0034] The concentration step may be effected at any convenient temperature, generally about 15° to about 60°C, and for the period of time to effect the desired degree of concentration. The temperature and other conditions used, to some degree, depend upon the membrane equipment used to effect the concentration and the desired protein concentration of the solution.

[0035] As is well known, ultrafiltration and similar selective membrane techniques permit low molecular weight species to pass therethrough while preventing higher molecular weight species from so doing. The low molecular weight species include not only the ionic species of the food grade salt but also low molecular weight

materials extracted from the source material, such as, carbohydrates, pigments and anti-nutritional factors, as well as any low molecular weight forms of the protein. The molecular weight cut-off of the membrane is usually chosen to ensure retention of a significant proportion of the protein in the solution, while permitting contaminants to pass through having regard to the different membrane materials and configurations.

[0036] Depending on the temperature employed in the concentration step, the concentrated protein solution may be warmed to a temperature of at least about 20°, and up to about 60°C, preferably about 25° to about 40°C, to decrease the viscosity of the concentrated protein solution to facilitate performance of the subsequent dilution step and micelle formation. The concentrated protein solution should not be heated beyond a temperature above which the temperature of the concentrated protein solution does not permit micelle formation on dilution by chilled water. The concentrated protein solution may be subject to a further defatting operation, if required, as described in US Patents Nos. 5,844,086 and 6,005,076.

[0037] The concentrated protein solution resulting from the concentration step and optional defatting step then is diluted to effect micelle formation by mixing the concentrated protein solution with chilled water having the volume required to achieve the degree of dilution desired. The concentrated protein solution is diluted by about 15 fold or less, preferably about 10 fold or less.

[0038] The chilled water with which the concentrated protein solution is mixed has a temperature of less than about 15°C, generally about 3° to about 15°C, preferably less than about 10°C, since improved yields of protein isolate in the form of protein micellar mass are attained with these colder temperatures at the dilution factors used.

[0039] In a batch operation, the batch of concentrated protein solution is added to a static body of chilled water having the desired volume, as discussed above. The dilution of the concentrated protein solution and consequential decrease in ionic strength causes the formation of a cloud-like mass of highly associated protein molecules in the form of discrete protein droplets in micellar form. In the batch procedure, the protein micelles are allowed to settle in the body of chilled water to form an aggregated, coalesced, dense, amorphous, sticky gluten-like protein micellar mass (PMM). The settling may be assisted, such as by centrifugation. Such induced settling decreases the

liquid content of the protein micellar mass, thereby decreasing the moisture content generally from about 70% by weight to about 95% by weight to a value of generally about 50% by weight to about 80% by weight of the total micellar mass. Decreasing the moisture content of the micellar mass in this way also decreases the occluded salt content of the micellar mass, and hence the salt content of dried isolate.

[0040] The settled isolate is separated from the residual aqueous phase or supernatant, such as by decantation of the residual aqueous phase from the settled mass or by centrifugation. The PMM may be used in the wet form or may be dried, by any convenient technique, such as spray drying, freeze drying or vacuum drum drying, to a dry form. The dry flax protein isolate has a high protein content, in excess of about 90 wt% protein, preferably at least about 100 wt% protein ($N \times 6.25$), and is substantially undenatured (as determined by differential scanning calorimetry). The dry flax protein isolate isolated from fatty oil seed meal also has a low residual fat content, when the procedures of USPs 5,844,086 and 6,005,076 are employed, which may be below about 1 wt%.

[0041] The supernatant from the PMM formation and settling step contains significant amounts of flax protein, not precipitated in the dilution step, and the supernatant may be processed to recover additional quantities of protein therefrom.

[0042] In such procedure, the supernatant from the dilution step, following removal of the PMM, may be concentrated to increase the protein concentration thereof. Such concentration is effected using any convenient selective membrane technique, such as ultrafiltration, using membranes with a suitable molecular weight cut-off permitting low molecular weight species, including the food grade salt and other non-proteinaceous low molecular weight materials extracted from the source material, to pass through the membrane, while retaining flax protein in the solution. Ultrafiltration membranes having a molecular weight cut-off of about 3000 to 10,000 daltons having regard to differing membranes and configurations, may be used. Concentration of the supernatant in this way also reduces the volume of liquid required to be dried to recover the protein, and hence the energy required for drying. The supernatant generally is concentrated to a protein content of about 50 to 300 g/L, preferably about 100 to about 200 g/L, prior to drying.

[0043] The concentrated supernatant may be dried by any convenient technique, such as spray drying, freeze drying or vacuum drum drying, to a dry form to provide a further flax protein isolate. Such further flax protein isolate has a high protein content, usually in excess of about 90 wt% protein ($N \times 6.25$), preferably at least 100 wt%, and is substantially undenatured (as determined by differential scanning calorimetry). If desired, the wet PMM may be combined with the concentrated supernatant prior to drying the combined protein streams by any convenient technique to provide a combined flax protein isolate. The combined flax protein isolate has a high protein content, in excess of about 90 wt% ($N \times 6.25$), preferably at least about 100 wt%, and is substantially undenatured (as determined by differential scanning calorimetry).

[0044] In another alternative procedure, a portion only of the concentrated supernatant may be mixed with at least part of the PMM and the resulting mixture dried. The remainder of the concentrated supernatant may be dried as any of the remainder of the PMM. Further, dried PMM and dried supernatant also may be dry mixed in any desired relative proportions.

[0045] By operating in this manner, a number of flax protein isolates may be recovered, in the form of dried PMM, dried supernatant and dried mixtures of various proportions by weight of PMM and supernatant, generally from about 5:95 to about 95:5 by weight, which may be desirable for attaining differing functional and nutritional properties.

EXAMPLES

Example 1:

[0046] This Example illustrates the removal of mucilage from Linola oil seed meal.

[0047] Linola oil seed was washed using varying concentration levels of sodium bicarbonate by mixing aqueous sodium bicarbonate with a seed: solvent ratio of 1:8 with Linola oil seed for one hour at 50°C using an overhead mixer set at high speed.

[0048] Washes were done at each concentration of aqueous sodium bicarbonate solution tested in order to compare the amount of mucilage recovered from the seeds. A total of 500 g of linola was washed in 4 L of sodium bicarbonate at each concentration.

[0049] The supernatant from each wash was decanted and 100 ml from each supernatant was diluted 1:1 with 88% ethanol to precipitate any solubilized mucilage. The mucilage then was collected and dried to calculate the total amount of mucilage removed from the seeds. The theoretical amount of mucilage in 500 g of Linola seed is 30 to 40 g.

[0050] The amounts extracted at the various concentration of sodium bicarbonate solution is shown in Table I:

TABLE I
Weight of mucilage removed during first wash at each concentration

Sodium bicarbonate concentration	Weight of mucilage (g)
0.1 M	16.0 g
0.3 M	16.4 g
0.5 M	32.0 g

[0051] As may be seen from Table I, a sodium bicarbonate concentration of 0.5 M is much more effective for removing mucilage than lower concentrations. In addition, at the 0.5 M concentration, the seed still had the slimy feel to it that is attributed to mucilage. A second, identical wash was done and another 34 grams of mucilage was removed. Following this second wash, a third wash was done and another 36.4 grams of mucilage was removed. A fourth wash yielded very little mucilage, indicating complete removal of mucilage from the 500 grams of linola seed. A total of 102.8 grams of mucilage was removed.

[0052] Following these washes, the seed did not have the "slimy" feel that mucilage imparts, providing another good indication that most of the mucilage had been removed.

Example 2:

[0053] This Example illustrates the preparation of a flax meal in accordance with one embodiment of the invention.

[0054] 25 kg of Linola oil seed, variety 2047, was added to 200 L of 0.5 M sodium bicarbonate at 50°C in a 400 L mixing tank. The slurry was stirred vigorously for one hour. After settling, the aqueous phase was decanted and the waste was discarded. A one-liter portion of the decanted aqueous phase was diluted with an equal

volume of ethanol, to precipitate mucilage to provide a rough estimate of the amount of mucilage recovered.

[0055] After decanting the aqueous phase, the seed was rinsed twice with hot tap water to remove any residual wash solution. The procedure of sodium bicarbonate extraction, separation and washing was repeated five times. The seed was then washed four times with hot tap water to remove any residual wash and mucilage. The seed was found to have lost its characteristic slimy feel, providing a good indication that the mucilage had been removed.

[0056] It was found that each successive aqueous sodium bicarbonate wash removed less mucilage than the previous one and that, by the fifth was, very little mucilage was precipitated from the one-liter portion of wash solution when diluted with ethanol, providing a good indication that most of the mucilage had been removed from the seed.

[0057] The seed then was dried, washed and defatted to remove the oil from the seed.

Example 3:

[0058] This Example illustrates the preparation of a flax protein isolate from mucilage-reduced meal by iso-electric precipitation.

[0059] 10 kg of the defatted linola oil seed meal, prepared as described in Example 2, was added to 200 L of 0.15 M NaCl solution at room temperature and the pH of the mixture was adjusted to 11.0 with 50 wt% sodium hydroxide solution. The slurry was stirred for one hour, after which the extracted meal was permitted to settle from the resulting protein solution for one hour.

[0060] 100 L of protein solution, having a protein content of 13 g/L, was then decanted and filtered through 20 and 0.2 μm filters in a filter press in order to clarify the solution. The clarified solution then was placed in a cooler at 4°C for 16 hours to permit any oil present to rise to the surface, where it could be skimmed off. Very little oil was seen, indicating a very effective defatting step.

[0061] The pH of the protein solution at ambient temperature was then adjusted to 4.0 using 3 N HCl and the protein immediately began to precipitate by a change of colour of the solution from a golden yellow colour to milky white. Once the mixing was

stopped, the protein precipitated rapidly from the solution. After a two-hour settling period, the supernatant was decanted and analyzed for protein content.

[0062] Following removal of supernatant, 10 L of pellet material was centrifuged at 10,000 xg for 5 minutes to decrease the residual supernatant content of the precipitated protein. The resulting pellet was reconstituted in 4 L of water and spray dried to provide 293 g of dried Linola protein isolate. The protein content of the spray dried protein was 101 wt% (N x 6.25) d.b.

Example 4:

[0063] This Example illustrates the functional properties of the Linola protein isolate produced in Example 3.

[0064] The Linola protein isolate produced according to the procedure of Example 3 (IEP Linola) was tested for functional properties of foaming and oil holding capacity in comparison to typical samples of PMM-derived and supernatant-derived canola protein isolates (CPI) produced according to the process described in pending US Patent Application No. 10/137,391 filed May 3, 2002 (WO 02/089597), assigned to the assignee hereof and the disclosure of which is incorporated herein by reference.

[0065] The test procedures employed are those set forth in copending United States Patent Application No. 10/137,306 filed May 3, 2002 (WO 02/089597), assigned to the assignee hereof and the disclosure of which is incorporated herein by reference.

[0066] The results obtained are set forth in Tables II and III below:

TABLE II
IEP Linola vs PMM-derived CPI

Batch	% Overrun (Foam Volume)	Foam Stability	Oil Holding Capacity (Ml oil/100 mg protein)	Globular size
CPI-1	1471.8	17.5	147.7	18.9
CPI-2	1030.4	32.7	190.5	29.1
CPI-3	1216.5	24.0	119.8	24.3
CPI-4	1051.2	45.3	115.4	21.6
CPI-5	1091.6	35.3	124.5	28.0
CPI-6	1196.1	34.0	166.9	24.6
IEP Linola Isolate	1770.9	0.67	118.9	59.0

TABLE III
IEP Linola vs Supernatant-derived CPI

Batch	% Overrun (Foam Volume)	Foam Stability	Oil Holding Capacity (Ml oil/100 mg protein)	Globular size
CPI-7	2603.6	22.7	67.2	72.6
CPI-8	1984.8	21.3	53.6	151.7
CPI-9	1924.4	22.0	43.3	151.7
CPI-10	1889.2	17.3	41.3	192.4
IEP Linola Isolate	1170.9	0.67	118.9	59.0

[0067] As may be seen from Table II, the linola protein isolate had superior foam properties to the PMM-derived canola protein isolates, with foam volume being higher and less drainage (better stability). The oil holding capacity of the linola protein isolate was comparable to the PMM-derived canola protein isolate but had a larger globular size.

[0068] As may be seen from Table III, the foam volume produced by the linola protein isolate was less than that produced by the supernatant-derived canola protein isolate, but the foam was much more stable. The linola protein isolate had superior emulsification properties to the supernatant-derived canola protein isolate. The oil holding capacity of the linola protein isolate was approximately twice as high as the supernatant-derived canola protein isolate and had a smaller globular size.

Example 5:

[0069] This Example illustrates the preparation of a flax protein isolate from mucilage-reduced meal by a micellar route.

[0070] 4 kg of the defatted linola oil seed meal, prepared as described in Example 2, was added to 80 L of 0.5 M NaCl solution at room temperature (5% w/v). The slurry was mixed for one hour, following which the slurry was allowed to settle for ½ hour and the aqueous protein solution decanted. The decanted aqueous protein solution had a protein content of 7.1 g/L and a volume of 55 L. The solution was filtered through 20 µm filter pads in a filter press to remove suspended solids. The press was flushed with 20 L of water to provide 75 L of a filtrate having a protein content of 5.28 g/L.

[0071] The filtrate was subjected to ultrafiltration using 300 daltons molecular weight cut-off ultrafiltration membranes to concentrate the solution to 1.3 L of concentrated aqueous protein solution (retentate) having a protein content of 174 g/L.

The retentate then was diluted into 9 volumes of 4°C water, which produced a white cloud of protein micelles.

[0072] A settling period of 16 hours was permitted after which the supernatant was decanted and centrifuged to recover as much of the precipitated material as possible to provide 11 L of supernatant having a protein content of 1.11 g/L. The Linola protein isolate pellet obtained from the precipitation step also was centrifuged to reduce its volume to a minimum level.

[0073] The Linola protein isolate pellet was dried to produce 81 g of dried protein, representing a 20 wt% yield of the protein extracted from the Linola seed meal. The dried Linola protein isolate had a protein content of 112 wt% (N x 6.25) d.b.

[0074] The clarified supernatant was concentrated using 300 daltons molecular weight cut-off membranes to 1.25 L of concentrated supernatant containing 63.3 g/L of protein. The concentrated supernatant was dried and produced 77 g of a Linola protein isolate (20% yield) having a protein content of 106 wt% (N x 6.25) d.b.

[0075] HPLC analysis, performed as described in copending United States Patent Application No. 10/413,371 filed April 15, 2003 (WO _____), assigned to the assignee hereof and the disclosure of which is incorporated herein by reference, of the two Linola fractions showed that each fraction is made up primarily of the same components, as may be seen in Figures 1 and 2. In both linola protein isolate fractions, the main protein component has a molecular weight of approximately 166,000 to 173,500 daltons with lesser components, one having a molecular weight in the range of 16,000 to 17,000 and another having a molecular weight of 420,000 to 440,000 daltons.

Example 6:

[0076] This Example illustrates the functional properties of the Linola protein isolates produced in Example 5.

[0077] The Linola protein isolates prepared according to the procedures of Example 5 were tested for functional properties of foaming and oil holding capacity, according to the procedures described in Example 4, in comparison to typical PMM-derived and supernatant-derived canola protein isolates (CPI) produced according to the process described in the aforementioned US Patent Application No. 10/137,391 (WO02/089597).

[0078] The results obtained are set forth in the following Tables IV and V:

TABLE IV

Linola vs. Canola PMM-derived CPI

Batch	% Overrun (Foam Volume)	Foam Stability	Oil Holding Capacity (ml oil/100 mg protein)	Globular size
CPI-1	1471.8	17.5	147.7	18.9
CPI-2	1030.4	32.7	190.5	29.1
CPI-3	1216.5	24.0	119.8	24.3
CPI-4	1051.2	45.3	115.4	21.6
CPI-5	1091.6	35.3	124.5	28.0
CPI-6	1196.1	34.0	166.9	24.6
Linola PMM-derived isolate	1470.0	0	81.4	11.8
Linola Supernatant-derived isolate	1464.0	2.0	139.6	19.8

TABLE V

Linola vs. Canola Supernatant-derived CPI

Batch	% Overrun (Foam Volume)	Foam Stability	Oil Holding Capacity (ml oil/100 mg protein)	Globular size
CPI-7	2603.6	22.7	67.2	72.6
CPI-8	1984.8	21.3	53.6	151.7
CPI-9	1924.4	22.0	43.3	151.7
CPI-10	1889.2	17.3	41.3	192.4
CPI-11	2776.8	4.0	47.1	118.9
Linola PMM-derived isolate	1464.02	2.0	139.5	19.8
Linola Supernatant-derived isolate	1470.0	0	81.4	11.8

[0079] As may be seen from Tables IV and V, the functional properties of the PMM-derived and supernatant-derived linola protein isolate were very similar, as might be expected from their similar HPLC properties, the main differences being in emulsion characteristics, differences lying between the two fractions in oil holding capacity and globular size.

[0080] In most categories, the functionality of the linola protein isolates was as good or better than the PMM-derived and supernatant-derived canola protein isolates. The linola protein isolate was weaker than the canola supernatant-derived isolate in foam volume, but the stability of the linola foam was much better.

SUMMARY OF DISCLOSURE

[0081] In summary of this disclosure, the present invention provided an improved method of producing a flax protein isolate in which mucilage first is extracted from the flax oil seed prior to flax oil removal and flax oil seed meal preparation,

enabling greater yields of protein isolates to be obtained and greater flexibility in isolation procedure to be achieved. Modifications are possible within the scope of the invention.

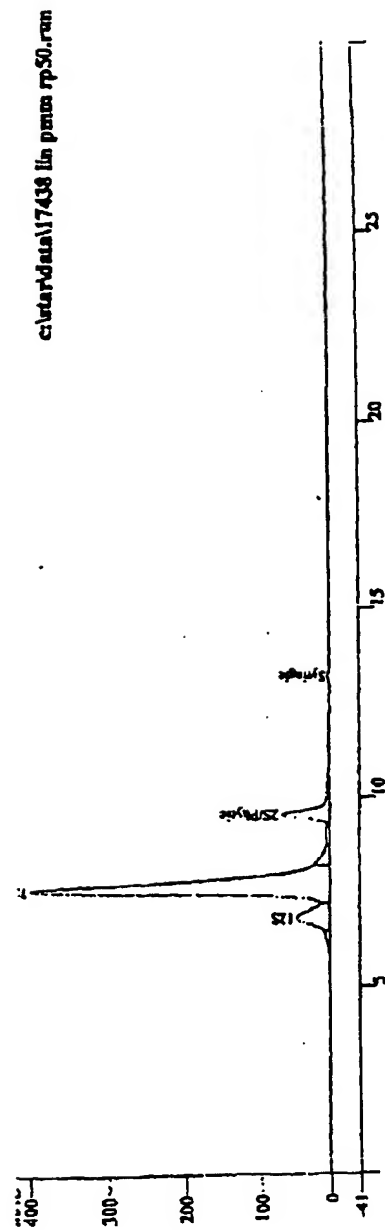


FIGURE 1. Linola PMM-Derived HPLC Chromatogram



FIGURE 2 – Linola Supernatant-derived HPLC Chromatogram

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